Evaluation of a Granulovirus (PoGV) and *Bacillus thuringiensis* subsp. *kurstaki* for Control of the Potato Tuberworm (Lepidoptera: Gelechiidae) in Stored Tubers

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J. Econ. Entomol. 101(5): 1540–1546 (2008)

ABSTRACT Liquid suspensions and dry formulations of a granulovirus (family *Baculoviridae*, genus *Granulovirus*, PoGV) derived from infected larvae and the bacterium *Bacillus thuringiensis* subsp. *kurstaki* (Berliner) (Btk) were evaluated for control of the potato tuberworm, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), in stored tubers. Laboratory bioassays at 25°C showed that both PoGV and a wettable powder (WP) formulation of Btk incorporated with carriers (water, talc, sand, diatomaceous earth, and kaolin clay), were effective against neonate larvae. Depending on the technique, 100% larval mortality was achieved at concentrations as low as 0.025 larval equivalents (LE) PoGV per kg tuber and 150 mg Btk WP per kg tuber. However, 100% mortality was never achieved with tests on preinfested tubers, ostensibly due to the higher dosage required to kill older instars inside tubers. The most effective PoGV formulations were dipping (water) and talc, with dipping most effective for postinfestation treatments, causing up to 91.6% mortality at 0.4 LE per kg. There was no significant effect of formulation in the Btk treatments. The protective effects of residues were also evaluated under longer-term storage conditions. Batches of tubers treated with PoGV or Btk via dipping (up to 0.1 LE and 150 mg WP per kg tuber) were stored in cages containing an initial potato tuberworm infestation (10% of tubers). Although potato tuberworm populations were reduced by up to 98.4% after 2 mo at 25°C, no treatments prevented the development and reproduction of the F1 generation. The sprouting of stored tubers seemed to be a limiting factor for sustained control. No significant treatment effects were detected in similar cages held at 12°C for 4.5 mo. Improved strategies for the application of PoGV and Btk for long-term potato tuberworm control in tuber stores, including the use of chemical sprout suppressants, are discussed.

KEY WORDS biopesticide, baculovirus, PoGV, *Solanum tuberosum*, pesticide residue

The potato tuberworm, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is a significant invasive pest of potatoes (*Solanum* spp.) in the tropics and subtropics (Radcliffe 1982), and it has recently become established in the Columbia Basin of the Pacific Northwest (Jensen et al. 2005). One concern is that moths may enter storage facilities from adjacent cull piles or waste material; alternatively, stores may become contaminated when infested tubers are bought into stores at harvest (Jensen 2006). In addition to direct damage, larval feeding galleries in tubers may encourage secondary pests or plant pathogens and severely reduce the quality and market value of a crop.

Currently, no insecticides are registered for postharvest use on potato tubers in North America. A granulovirus of potato tuberworm (family *Baculoviridae*, genus *Granulovirus*, PoGV) has been isolated in several countries, including Australia (Reed 1969), Indonesia (Zeddam et al. 1999), Peru (Alcázar et al. 1991), the Republic of Yemen (Kroschel et al. 1996), and California in the United States (Hunter et al. 1975). Larvae infected with PoGV typically complete larval development but fail to pupate, thus preventing development of future generations (Sporleder et al. 2005). A dry powder formulation '*Phthorimaea* Baculovirus' containing PoGV occlusion bodies incorporated into talc (magnesium silicate) has been successfully developed and promoted by the International Potato Center (CIP) in Lima, Peru, as a nontoxic method to protect tubers in nonrefrigerated storage facilities in several developing countries (Lagnaoui et al. 1997, Wraight et al. 2007). Dry formulations containing the bacterium, *Bacillus thuringiensis* Berliner (Bt), also have been tested for potato tuberworm control in nonrefrigerated stores with some success (Lal 1987, Hamilton and Macdonald 1990, Kroschel and Koch 1996, Salama and Salem 2000). The dark and dry conditions in tuber storage houses are amenable for PoGV and Bt spores and crystals, which are rapidly inactivated in the field due to UV irradiation, temper-

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ature, wind, and rainfall (Kroschel et al. 1996). In the absence of such interventions, initial potato tuberworm infestations may develop quickly, especially in nonrefrigerated stores, spread to additional tubers, and destroy an entire crop within 2–4 mo (Raman et al. 1987, von Arx et al. 1987).

In the current study, we compared various dry and liquid formulations of PoGV and Btk to control potato tuberworm at different stages of development on tubers. The most effective treatments were further tested in a longer term simulated storage trial at two temperature gradients.

Materials and Methods

Potato Tuberworm Colony. Our colony originated from a field population collected in potato fields at Oregon State University's Hermiston Agricultural Research and Experiment Station in 2004. The colony is reared on tubers ('Russet Burbank') following the procedure described previously (Sporleder et al. 2004, 2005).

Virus Production. The PoGV isolate used in our studies was derived from a dry formulation originally produced by CIP and imported from Ecuador under permit 29878. For in vivo production, filter papers containing potato tuberworm eggs were inoculated with virus suspensions at a rate of one virus-infected fourth-instar larval equivalent (LE) per ml. In addition, tubers used to feed the eclosing neonates also were inoculated through dipping at a rate of 1 LE per 100 ml, and air-dried before infestation. Infected larvae were collected 21–28 d after infestation and frozen until use in further virus production or for experiments. Fresh batches of virus were produced approximately once per month. Based on quantification of occlusion body (OB) density by using the methods outlined by Sporleder et al. (2005), we estimated that each infected fourth instar contained on average 2.3×10^{10} OBs.

Preparation of Freeze-Dried Formulations. Dry formulations of PoGV were prepared using modifications of the methods described by CIP (1992). In our procedure, 20 virus-infected fourth-instar larvae were macerated in 10 ml of sterile water with a glass tissue grinder, and the homogenate was diluted in 250 ml of sterile water and a dispersing agent [Silwet L77 at 0.05% (vol:vol), Loveland Industries, Inc., Greeley, CO]. Virus suspensions (100 ml) containing 80, 20, 5, 1.25, and 0 (control) LE per liter plus 0.05% Silwet were prepared through serial dilution. PoGV suspensions were incorporated into inert dry "carriers." Materials tested were talcum powder (Fisher, Fair Lawn, NJ), diatomaceous earth (DE) (MP Biomedicals, Solon, OH), kaolin clay (Cocoon, Advan LLC, Roswell, GA), and masonry sand (Central Pre-Mix Concrete Co., Yakima, WA), sieved to remove particles >1 mm. Five milliliters of suspension for each PoGV concentration was added to each material in an 80-ml glass beaker at the rate of 100% (wt:vol) (talc and kaolin), 40% (wt:vol) (DE), and 30% (wt:vol) (sand). Because preliminary tests showed DE and sand had reduced adherence to tubers compared with talc and kaolin, higher virus concentrations were prepared with these materials in an attempt to standardize the quantity of active ingredient (AI) applied in the bioassay. Each sample was mixed into a paste (except sand which did not dissolve) with a glass stirring rod and the beaker covered with two layers of Kimwipe (Kimberly Clark, Irving, TX). Samples were frozen at −80°C for 2 h and placed overnight in a Freeze-Dry System (Stoppering Tray Dryer model, Labconco Corp., Kansas City, MO). Previous studies showed lyophilization did not reduce the virulence of the nucleopolyhedrovirus of *Autographa californica* (Speyer) (Hughes and Wood 1996) or the granulovirus of *Zeiraphera diniana* Guenée even after 8-mo storage at 2°C (Schmid 1974). All samples were removed under a fume hood into 20-ml glass vials. To improve coverage, a food grade flow conditioning silica used as an anticaking agent (Flo-Gard, PPG Industries, Inc., Monroeville, PA) was added to all formulations (except sand) at 1% (wt:wt).

A similar procedure was used to produce lyophilized formulations of Btk. A commercial strain for Lepidoptera formulated as a wettable powder (WP) (Deliver, Certis USA, Columbia, MD) was used. In this case, suspensions containing 120, 30, 7.5, 1.875, and 0 g WP/liter plus 0.05% Silwet were incorporated into carriers at the same rates (wt:vol) as PoGV. Prepared samples were refrigerated and used within 48 h.

Comparisons of Formulations and Effect of Pest Stage Targeted. Because storage studies need to be conducted over extended periods, preliminary bioassays were conducted with individual tubers to screen various treatments. In total 1,600 tubers (Russett Burbank) were used in the study. On test days, 100 tubers free of pesticide residues and weighing 100–200 g were washed, air-dried, and randomly allocated among 25 treatments, i.e., five carriers (described above) each prepared with five concentrations of virus or Btk (i.e., four tubers per treatment). Tests were conducted weekly. Due to the number of treatments, PoGV and Btk were tested on separate dates, although alternated to minimize experimental bias in materials or procedures. Tubers were weighed and treated at the rate of 0.5% (wt:wt) (talc and kaolin), 0.2% (wt:wt) (DE), or 0.15% (wt:wt) (sand). This procedure delivered concentrations of 0.4, 0.1, 0.025, 0.00625, and 0 LE/kg tuber (PoGV) and 600, 150, 37.5, 9.375, and 0 mg of Btk WP/kg tuber. To apply treatments, tubers were gently shaken for 2 min in sealed plastic bags (4-liter capacity). As an additional treatment, tubers were dipped in aqueous suspensions of PoGV and Btk that delivered equivalent concentrations of virus or Btk WP (based on a mean deposition of 10 ml liquid/kg tuber). To achieve this, tubers were individually dipped in 1-liter beakers containing 40, 10, 2.5, 0.625, and 0 LE per liter (PoGV) and 60, 15, 3.75, 0.9375 and 0 g Btk WP/liter, plus 0.05% (vol:vol) Silwet, and allowed to air dry.

For assessments, treated tubers were placed individually in 0.47-liter plastic cups containing 30 g of sand as a pupation substrate. A 6.4-cm-diameter hole in the lid covered with polyester mesh provided ven-

tilation. Each tuber was infested with 20 potato tuberworm eggs cut out from filter papers and pinned onto treated tubers. Neonates emerged within 24–72 h; 10 ml of water was added to the sand in cups to maintain high humidity to facilitate hatching. Emerged moths (or healthy pupae) and sick or dead larvae were evaluated after 28 d at 25°C. Because some of the dry carriers may act as desiccating agents, tubers were also reweighed to compare weight losses during incubation (the weights of any carriers were subtracted for analysis). In addition to the above-mentioned procedure (i.e., preinfestation treatment), the entire procedure was repeated with tubers that were already infested with second- or third-instar larvae (i.e., postinfestation treatment). In this case, tubers were infested with 20 eggs (as described above) 8 d before treatment. Each test was repeated with fresh materials, and each test date (i.e., mean of four tubers) was considered a replicate for analysis ($n = 4$).

Reduced Rate PoGV Study. Because even the lowest rates of PoGV in tests described above were highly effective as preinfestation treatments (i.e., against neonates), an additional study was conducted to describe the dosage–mortality relationship (Finney 1971). In this case, groups of five tubers were each dipped in a range of nine concentrations that were applied between 0.14 and 1.90×10^{-6} LE per kg. Treatments were evaluated as described above.

Simulated Store Trial. The protective effects of PoGV and Btk residues against potato tuberworm infestations were evaluated under longer term storage conditions. Based on the previous study, dipping was chosen due to its effectiveness and ease of application. Treatments included the lowest two dosages of PoGV and Btk WP that were also highly effective, i.e., caused >90% mortality of neonates. Based on the experimental design, we hypothesized that such treatments would prevent the generation of F1 pupae/adults and limit the spread of an initial infestation to surrounding tubers.

In total, 1,500 tubers (Russet Burbank) were used in the study. Treatments consisted of PoGV (0.625 and 10 LE per liter, which applied 0.00625 and 0.1 LE per kg tuber), Btk (3.75 and 15 g/liter, which applied 37.5 and 150 mg/kg), and controls. Silwet T was always included at 0.05% (vol:vol). Each replicate (30 tubers) was dipped in a separate suspension inside a 2-liter beaker. Once thoroughly dry, treated tubers were placed inside a cylindrical plastic cage (20 liters) containing 300 g of sand at the base and ventilated with two 10-cm-diameter holes covered with polyester mesh on the sides. To provide an initial source of potato tuberworm, three tubers from each replicate were infested with 20 eggs, as described previously, 8 d before treatment (i.e., 10% initial infestation). Infested tubers were positioned at the base of cages, with remaining tubers placed around and on top. There were five replicate cages per treatment and PoGV and Btk were treated on different days. Cages were sealed to prevent escape and incubated in the dark at 25°C for 2 mo. Cages were periodically examined, and the proportion of infested tubers and number of pupae and moths were assessed at the end of the study. Due to the high potato tuberworm populations generated in control cages, second generation moths were removed and counted starting after 42 d of storage. To provide conditions approximating some commercially refrigerated warehouses, the study was repeated with the exception that cages containing treated tubers were stored at 12°C. Because potato tuberworm development was considerably reduced at this temperature, the study was continued for ≈4.5 mo.

Data Analysis. Univariate analysis of variance (ANOVA) was used to assess treatment main effects and interactions with significant F ratios ($P < 0.05$) separated with Fisher least significant difference (LSD) for multiple comparisons (SAS Institute 2001). All proportional and count data were normalized via arcsine and log (n + 1), respectively, before analysis. The reduced rate bioassay was analyzed based on probit (normal sigmoid) comparisons of the LC_{50} and LC_{95} values.

Results

Comparisons of Formulations and Effect of Pest Stage Targeted. An ANOVA model describing larval mortalities showed that the potato tubermoth stage targeted was a highly significant factor for both PoGV ($F_{1,150} = 1133$; $P < 0.0001$) and Btk ($F_{1,150} = 298$; $P < 0.0001$) treatments and also entered in significant interactions with formulation carrier for both PoGV ($F_{4,150} = 15.2$; $P < 0.0001$) and Btk ($F_{4,150} = 5.8$; $P < 0.0001$). To understand the nature of these interactions and allow the most effective treatments to be assessed under different circumstances, we compared PoGV and Btk treatments separately according to the pest stage targeted.

Preinfestation treatments (targeting neonates) were always more effective compared with equivalent treatments applied postinfestation (Tables 1 and 2). Depending on the treatment, 100% mortality of neonates was achieved at concentrations as low as 0.025 PoGV LE per kg tuber and 150 mg of Btk WP per kg tuber. By contrast, 100% mortality was never achieved when infested tubers were treated, although there was a clear dosage–mortality response, and higher concentrations of both agents may have been more effective. The most effective carriers for PoGV were dipping and talc; dipping was also the most effective postinfestation treatment (Table 1). In Btk treatments, there were no significant differences among formulation carriers, although increased variability among response was noted, and further replication may have been required to reveal statistical differences (Table 2). Some carriers on their own (notably talc and DE) also provided significant control of neonates, compared with dipped treatment that represented a "no residue" comparison, but they were not effective against larvae already inside tubers (Tables 1 and 2).

Infected fourth-instar larvae (normally already dead) were commonly found inside cocoons in sand at the base of cups in PoGV treatments. These ac-

Table 1. Mean (SEM) percentage of mortality of potato tuberworm on potato tubers treated with different concentrations of PoGV applied in dry carriers or by dipping tubers in aqueous suspensions

Concn (LE/kg tuber)	Formulation carrier[a]				
	Dipped	Talc	Sand	DE	Kaolin
Preinfestation treatment (target neonates)					
0	14.7 (1.3)Bc[b]	70.6 (9.5)Ba	18.8 (4.0)Cc	54.7 (9.2)Cab	37.2 (5.9)Dbc
0.00625	99.7 (0.3)Aa	99.7 (0.3)Aa	87.2 (8.0)Bb	97.5 (0.9)Bab	91.6 (0.6)Cb
0.025	99.4 (0.4)A	100.0 (0.0)A	98.4 (1.2)A	100.0 (0.0)A	98.4 (0.8)B
0.1	100.0 (0.0)A	100.0 (0.0)A	99.7 (0.3)A	100.0 (0.0)A	100.0 (0.0)A
0.4	100.0 (0.0)A	100.0 (0.0)A	99.7 (0.3)A	100.0 (0.0)A	100.0 (0.0)A
Postinfestation treatment (second/third instars inside tubers)					
0	15.6 (6.1)C[b]	15.3 (3.7)D	17.2 (3.4)D	14.4 (4.4)D	22.8 (6.5)C
0.00625	78.4 (5.4)Ba	56.3 (2.4)Cb	35.6 (3.4)Cc	38.1 (3.6)Cc	50.9 (9.7)Bbc
0.025	88.1 (5.0)ABa	73.4 (6.5)Bb	44.7 (6.9)Cc	53.1 (3.7)Bc	51.9 (5.1)Bc
0.1	89.7 (5.3)ABa	80.6 (2.3)ABb	65.9 (1.6)Bc	63.8 (4.4)Bc	64.1 (1.1)Bc
0.4	91.6 (3.5)A	88.8 (3.4)A	83.1 (2.9)A	81.6 (3.2)A	82.5 (4.7)A

Effectiveness of treating tubers both before and after infestation was compared.

[a] Applied to tubers at 0.15–0.5% (wt:wt) (see Materials and Methods).

[b] Letters represent differences analyzed separately for pre- and postinfestation treatments (Fisher's LSD test at $P < 0.05$). Uppercase letters show differences between virus concentrations for a given carrier; lowercase letters show differences between carriers for a given concentration (rows). The lack of some lowercase letters indicates formulation was not significant at those concentrations. Mean of four replicate tests.

counted for 40.4% of the potato tuberworm mortality compared with only 3.3% among Btk treatments (pooled across treatments). The remaining proportion of larvae presumably died inside tubers; in the case of Btk, likely at an early stage of development. Only 1.1 and 2.3% of the total mortality found in the PoGV/Btk and control treatments (pooled) occurred in the pupal stage. Compared at the highest concentrations (that minimized larval feeding on tubers), there was no effect of formulation carrier on percentage of weight lost by tubers during incubation, which ranged from 8.2% (talc) to 11.1% (sand) ($F_{4,74} = 1.2$; $P = 0.34$). Interestingly, analysis of the full data set revealed carrier was a significant factor for tuber weight loss ($F_{4,385} = 3.8$; $P < 0.005$), which would account for the differential effect of tuber consumption by larvae surviving various treatments (Tables 1 and 2).

Reduced Rate PoGV Study. Based on the bioassays, six concentrations that produced mortalities between 14 and 94% were selected for probit analysis. This yielded an LC_{50} (± 95% confidence interval) value of 2.43×10^{-4} ($1.68–3.33 \times 10^{-4}$) and LC_{95} value of 3.69×10^{-3} ($2.22–7.91 \times 10^{-3}$) LE per kg tuber, with a χ^2 value of 5.40 (df = 4), $P = 0.25$, and a moderate regression slope of 1.39 (SE = 0.16).

Simulated Store Trial. At 25°C, potato tuberworm developed rapidly. Moths emerging from the original infestation were observed after 15 d and F1 adults after 42 d inside control storage cages. Compared with controls, PoGV and Btk treatments reduced the final number of potato tuberworm adults in cages by up to 98.4%

Table 2. Mean (SEM) percentage of mortality of potato tuberworm on potato tubers treated with different concentrations of Btk applied in dry carriers or by dipping tubers in aqueous suspensions

Concn (mg/kg tuber)	Formulation carrier[a]				
	Dipped	Talc	Sand	DE	Kaolin
Preinfestation treatment (target neonates)					
0	17.5 (5.9)Cc[b]	74.4 (4.1)Ca	13.8 (4.4)Cc	49.4 (6.5)Cb	40.6 (6.9)Cb
9.375	72.2 (12.0)B	88.8 (9.6)B	86.3 (7.5)B	96.6 (2.6)B	73.4 (13.1)B
37.5	92.2 (6.2)A	99.1 (0.3)AB	97.2 (1.1)AB	99.1 (0.9)AB	87.2 (7.9)AB
150	99.7 (0.3)A	100.0 (0.0)A	99.4 (0.6)A	100.0 (0.0)A	97.5 (2.1)A
600	100.0 (0.0)A	100.0 (0.0)A	100.0 (0.0)A	100.0 (0.0)A	100.0 (0.0)A
Postinfestation treatment (second/third instars inside tubers)					
0	18.1 (4.2)D[b]	24.1 (1.2)C	18.4 (4.0)C	17.1 (3.5)C	14.7 (1.7)C
9.375	51.9 (8.4)C	34.7 (8.1)BC	32.5 (6.5)C	45.3 (7.0)B	23.8 (3.9)C
37.5	73.1 (8.9)BC	54.7 (10.3)B	63.4 (5.0)B	59.7 (9.2)B	38.1 (7.2)C
150	84.4 (8.6)B	84.4 (6.5)A	90.3 (4.8)A	81.3 (8.7)A	63.1 (12.5)B
600	98.8 (0.5)A	93.8 (3.1)A	94.7 (3.3)A	92.8 (3.2)A	89.4 (6.9)A

The effectiveness of treating tubers both before and following infestation was compared.

[a] Applied to tubers at 0.15–0.5% (wt:wt) (see Materials and Methods).

[b] Letters represent differences analyzed separately for pre- and postinfestation treatments (Fisher's LSD test at $P < 0.05$). Uppercase letters show differences between Btk concentrations for a given carrier, and lowercase letters show differences between carriers for a given concentration (rows). The lack of most lowercase letters indicates formulation was not significant at most concentrations tested. Mean of four replicate tests.

Table 3. Effects of PoGV and Btk treatments on potato tuberworm development and tuber infestation in simulated storage study at two incubation regimes

Treatment (dipped)	Inoculum/kg tuber	After 2 mo at 25°C[a]		After 4.5 mo at 12°C[a]	
		No. moths and pupae[b]	% Infestation[c]	No. moths[b]	% Infestation[c]
Control		1,073.0 (101.4)a[d]	100.0 (0.0)a	18.4 (3.0) N.S.	27.0 (3.7) N.S.
PoGV	0.1 LE	17.0 (1.9)d	92.6 (6.4)a	26.2 (4.3)	23.9 (3.5)
	0.00625 LE	55.8 (3.6)c	98.8 (0.7)a	14.6 (3.1)	30.7 (3.7)
Btk	150 mg	52.4 (4.0)c	77.6 (6.8)b	15.0 (1.8)	18.8 (2.6)
	37.5 mg	237.0 (22.2)b	99.4 (0.6)a	12.6 (3.6)	17.6 (3.1)

[a] Mean (SEM) from five storage cages (30 tubers per cage).
[b] Composed of F1 progeny at 25°C only.
[c] Initially 10% infested.
[d] Letters indicate differences following one-way ANOVA; Fisher's LSD at $P < 0.05$.

after 2 mo, although were slightly less effective at the lower concentrations tested (Table 3). Unfortunately, none of the treatments eliminated the development of F1 pupae and moths or prevented high larval infestation of tuber from occurring; however, the higher rate of Btk significantly reduced percentage of infestation compared with controls.

At 12°C, no significant effects of treatments were detected (Table 3). The slow development of potato tuberworm at the lower temperature did not allow us to fully quantify the effect of treatments because F1 pupae and moths had not yet been recovered (in control cages) after 4.5 mo storage, at which time tubers had begun to decay. Development of pupae and moths from the original infestation was not observed until days 46 and 74 of storage. It also was noted that the numbers of dead moths (which must have originated from initial infestation) were not reduced in PoGV and Btk treatments, possibly due to reduced feeding at the low temperature. However, background mortality was also much higher at 12°C compared with 25°C, i.e., only 31% of infested larvae produced moths in controls (Table 3) compared with ≈83% at 25°C (Tables 1 and 2). Percentage of tuber infestation increased 2–3-fold in all treatments at 12°C, possibly due to feeding of the F1 larval generation. Although not quantified, potato tuberworm eggs were frequently found deposited on tubers during assessments.

Discussion

Our initial studies showed that both PoGV and Btk applied to tubers via various dry formulations or by dipping with aqueous suspensions (tubers could also be sprayed) provides methods to control potato tuberworm in storage. However, we noted that preinfestation (i.e., protective) treatments were more effective compared with postinfestation (i.e., curative) treatments for larvae already inside tubers. This difference in effectiveness is most likely explained by the higher dosage required for older larvae compared with neonates penetrating treated surfaces. Based on leaf-disc bioassays, Sporleder et al. (2007) estimated the LC_{50} value of PoGV increased >1,000-fold from neonate to 9-d-old larvae, increasing in a log linear manner with both body weight and physiological age. As documented for other Lepidoptera (Glare and O'Callaghan 2000), laboratory tests by Salama et al. (1995) revealed the susceptibility of potato tuberworm larvae to Bt toxins also declined significantly during their development. Moreover, larvae that are already present inside tubers may not be exposed to surface treatments until fully grown when exiting tubers, and they may potentially avoid consuming residues.

The treatment of tubers with microbial pesticides may thus be best used to prevent the spread of an initial infestation by eliminating further breeding, or to prevent moth establishment, within a store. Early control of potato tuberworm is critical because there is currently zero level of tolerance for damage in processed potatoes in eastern Washington/Oregon (Rondon et al. 2006). After tests of a range of materials, Kroschel and Koch (1996) suggested that control of potato tuberworm in storage can only be considered effective when no more live moths remain after 3 mo of storage and damage to tubers is slight or nonexistent. Thus, even under ideal conditions, the slow speed of kill of microbials (especially PoGV) potentially represents a practical limitation.

Unfortunately, tubers were not adequately protected in our storage studies. Although we anticipated some reproduction from the initial 10% infestation (at 25°C between 6.2 and 16.1 moths per cage were expected to survive based on the treatments selected), we hypothesized that the requirement for F1 neonates to penetrate tubers would prevent further successful development (at least at the higher concentrations tested), thus eliminating the infestation. The reasons for the failure are unknown, although we suspect that tuber sprouts (often noted after several weeks of storage and occasionally removed) created new untreated areas and enabled some neonates to bypass residues. Potato tuberworm neonates commonly enter tubers in the vicinity of the tuber eyes (Gurr and Symington 1998); thus, they may similarly gain entry via new shoot growth. Das et al. (1992) also reported that a talc formulation of PoGV in nonrefrigerated store trials was quite successful up to 2 mo, but it became less effective as sprouts were attacked by larvae. Although less obvious, the dislodging of residues by moths, such as during oviposition-related behaviors, or the exit

holes created by previous larvae could also conceivably create untreated areas for neonates to penetrate. It is unlikely that inactivation of residues per se was an issue under the relatively dark conditions in which tubers were held.

Our storage study also suggested that, depending on the period stored, treatments may not be required for tubers maintained under temperature conditions at which potato tuberworm development is seriously curtailed. At 10.6 and 16.1°C, larval development may take 106.7 and 58.1 d, respectively, compared with 16.7 d at 25°C (Sporleder et al. 2004). In the United States, most tuber storage facilities are refrigerated, with final temperatures in the range 3–13°C, depending on the purpose and cultivar of the tubers (Knowles and Plissey 2007). However, large warehouses may require several weeks to achieve temperature stabilization (temperatures are reduced slowly to facilitate curing and wound healing), thus providing a significant window for pest development (Knowles and Plissey 2007). In addition, metabolic heat associated with microbial activity may (e.g., tuber rotting) may provide areas of elevated temperature in which potato tuberworm is able to develop (L.A.L., unpublished data).

PoGV is not currently produced in North America or Europe, and it is unclear what formulation methods and application rates of virus would be considered practical. The talc-based *Phthorimaea* Baculovirus containing 20 LE per kg has been successfully used in small-scale nonrefrigerated "rustic" stores in several countries in the Andes, Middle East, northern Africa, and Asia (Wraight et al. 2007). The method most commonly used is to shake tubers in bags or buckets containing powder preparations at the rate of 5 g/kg tuber (0.1 LE per kg tuber) (CIP 1992). Dry formulations of Bt also have previously controlled potato tuberworm in nonrefrigerated stores (Lal 1987, Hamilton and Macdonald 1990, Salama and Salem 2000). Kroschel and Koch (1996) showed that Btk (Dipel) combined with fine sand dust containing quartz and applied dry at 0.1% (wt:wt) (300 mg product per kg tuber) tuber was very effective, killing 100% neonates, 96% of second-stage larvae (L2) inside tubers, and providing up to 98% control after 3 mo. Interestingly, Kroschel and Koch (1996) found sand alone provided 94.6% control of neonates, which was not noted in our study, possibly due to the less abrasive sand used in our studies.

Although talc and sand were included in our studies, we showed that other relatively inexpensive materials may be used as carriers. DE has received significant interest as an alternative to synthetic residual insecticides for beetles in grain stores and is thought to work by imposing mechanical disruption and physiological stress (desiccation) to young instars (Korunic 1998). However, our studies suggest that DE alone will only provide partial suppression of potato tuberworm under the humid conditions expected inside a potato store. Similar results were observed for kaolin clay, which is marketed as a crop sunburn protectant. It was noted that dipping tubers with aqueous suspensions, which ostensibly allows some penetration into larval galleries (the wetting agent was likely beneficial) was the most effective postinfestation treatment for PoGV. Dipping also has the advantage of leaving no additional residues that may pose health hazards; however, washed tubers may store less well (Knowles and Plissey 2007).

In practical terms, integrated control of potato tuberworm in both field and storage is also important to minimize storage losses (von Arx et al. 1987, Fuglie et al. 1993, Lagnaoui et al. 1997, Kroschel and Sporleder 2006). Various cultural controls, including pheromone trapping to detect infestations, good sanitation, and early planting may help reduce postharvest infestations. Repellant materials, such as dried leaves of lantana and eucalyptus, also have been successfully used to suppress potato tuberworm in nonrefrigerated stores for 4–6 mo (Lal 1987, Raman et al. 1987). The possibility of using the endophytic fungus *Muscodor albus* as a biofumigant to control potato tuberworm in storage also has been studied (Lacey and Neven 2006, Lacey et al. 2008).

In conclusion, PoGV and Btk provide options for long-term control for potato tuberworm in storage. Further scaled up storage studies are planned using improved air circulation and possibly fungicides to minimize rotting, and chemical sprout suppressants (such as chlorpropham) to help maintain effective residue coverage and tuber quality over longer periods of storage. Optimization of microbial pesticides to improve application to large storage conditions, such as using ultralow volume application, may be desirable, although this technology has yet to be evaluated.

Acknowledgments

We thank Heather Headrick, Dana Jones, and Belinda Bray Bishop for technical assistance and our cooperators Andrew Jensen at the Washington State Potato Commission and Jürgen Kroschel, Marc Sporleder, and Birgit Schaub at the International Potato Center. Rob Fritts, Jr. (Advan LLC) kindly supplied materials for our studies. Don Hostetter and Dave Horton provided helpful manuscript reviews. Financial support was provided by the Washington State Potato Commission, the USDA–National Potato Council and Washington State Commission on Pesticide Registration.

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Received 29 February 2008; accepted 29 May 2008.